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Transmission of Smallpox by Contact and by Aerosol Routes in *Macaca irus**

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Smallpox is believed not to occur naturally in species other than man. However, reports of several epizootics of an exanthematous disease, similar to smallpox, in wild monkeys have raised the question of a simian reservoir. If such a reservoir for smallpox exists, the eradication of this disease from the world would be a difficult or impossible task. Transmission of smallpox in Macaca irus *has been studied to determine whether transmission occurs and if infection chains can be maintained by this species.*

Transmission was consistently accomplished by both contact and aerosol routes. In the contact transmission studies, the smallpox infection was maintained through 6 passages but was lost with the seventh passage. The virulence of the virus did not appear to increase as the virus was serially passed in monkeys. Continuing studies of the possible occurrence of smallpox and of monkeypox in simian populations are warranted.

At present there is no evidence of naturally occurring variola infection in animal species other than man. However, the threat to eradication programmes of a simian reservoir has prompted a re-evaluation of smallpox in monkeys.

Anderson (1861) described a vesicular exanthem infecting monkeys in Panama in 1841 and Bleyer (1922) reported a similar outbreak in *Cebus* monkeys in Brazil in 1922. Both epizootics caused widespread death in the monkey population and preceded, or were coincident with, smallpox epidemics in nearby villages. Laboratory confirmation of variola or monkeypox infection in the wild monkeys was not possible in these 2 outbreaks.

The pathogenesis of smallpox infection in monkeys has been well documented. Magrath (1904) and Hahon (1961) reviewed the culturing of human smallpox specimens in monkeys during the 19th and early 20th centuries. Hahon & Wilson (1960), Westwood et al. (1966) and Lancaster et al. (1966) studied the histopathogenesis of variola in monkeys and isolated variola virus from the lungs and nasal washings of experimentally infected animals. The transmission of smallpox from infected to control animals was studied in a limited number of experiments by Hahon & McGavran (1961), Blaxhall (1930) and Herrlich (personal communication); no cross infections were observed.

The following studies were conducted to determine whether transmission of smallpox can occur and be maintained among susceptible monkeys for more than 1 or 2 generations of disease and to define the mode of transmission.

METHODS

Monkeys

Cynomolgous monkeys, *Macaca irus philippinensis*, were used in all studies. Altogether, 22 animals received in 2 shipments from Cebu Island, Philippines, were introduced to the study after a quarantine period of 6 weeks. No evidence was seen of a vesicular exanthem suggestive of monkeypox infection in the quarantined animals. A " squeeze rack " primate cage[1,2] was used for the contact transmission studies.

Virus strain

The strain of variola major virus used in all studies (the Harvey strain) was isolated from a

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[1] Produced by Harford Metal Products Inc., Aberdeen, Md., USA.

[2] The use of trade names is for identification only and does not constitute endorsement by the Health Services and Mental Health Administration or by the US Department of Health, Education, and Welfare.

smallpox patient in India and was kindly provided by Professor Keith Dumbell, Wright Fleming Institute, London. It had undergone 3 chicken embryo passages and contained 2.5×10^6 pock-forming units per ml when titrated on the chorioallantoic membrane of 10-day-old chicken eggs.

Studies

In the first contact study, 4 monkeys were inoculated intranasally with 2 ml of virus suspension and quarantined for 48 hours in an isolated laboratory. They were then placed in separate cages, each with a healthy control animal; all the monkeys were inspected daily. Rectal temperatures were taken daily during the course of illness in the inoculated monkeys and until the onset of fever or lesions in the controls. Control monkeys which developed fever or lesions were removed and isolated in separate clean cages and new healthy control animals were placed with them. These new control animals were then observed daily for temperature changes and evidence of smallpox infection.

The second contact study was conducted in the same manner as the first; however, only 1 animal (No. 36) was inoculated initially. Each animal that contracted the disease was then isolated with a new control animal until smallpox transmission ceased.

In the next 2 studies, aerosol transmission of variola was investigated. A polyethylene isolation chamber 70 inches by 43 inches by 25 inches (approximately 178 cm × 109 cm × 64 cm) (Fig. 1) was used. Intake and exhaust air was filtered through Cambridge absolute filters. Smoke testing confirmed a unidirectional flow of air at a rate of 2.6 ft^3 (0.072 m^3) per minute. Modified primate-restraining chairs held the animals in the chamber. Each animal was inoculated intranasally with variola virus suspension and placed in the aerosol chamber at the intake (upwind) end. After a period of 72 or 96 hours, a control animal was placed at the outlet (downwind) end of the chamber, 36 inches (approximately 1 m) away from the infected animal. Rectal temperatures of both animals were taken daily. All food, water and equipment for each animal was kept separate, and at no time did the animals have physical contact with each other. They were maintained in the aerosol chamber until fever or lesions were noted in the control animal.

Collection of specimens

Three serum specimens were obtained from each animal. The first was taken prior to exposure; the second was taken when fever or rash developed; and the third at the termination of the study, 12–16 days

FIG. 1
AEROSOL EXPOSURE CHAMBER WITH PRIMATE-RESTRAINING CHAIRS USED IN THE SMALLPOX AEROSOL TRANSMISSION STUDIES

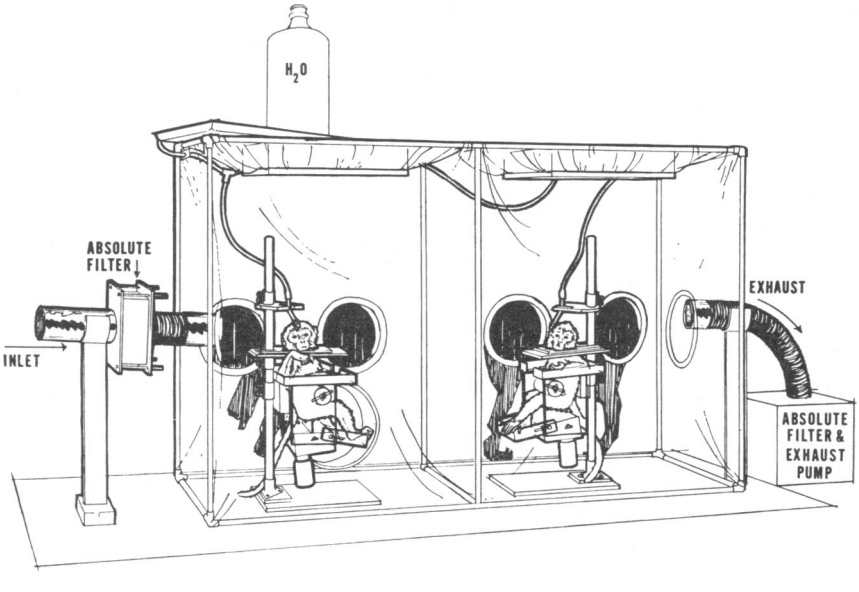


TABLE 1

DISTRIBUTION OF SMALLPOX LESIONS IN 8 *MACACA IRUS* INFECTED IN CONTACT AND AEROSOL TRANSMISSION STUDIES

Location of lesion	No. of monkeys								Number of lesions	Percentage distribution
	37	42	60	62	63 [a]	64	70	71		
Head		1		1				3	5	3.0
Face					1		2		3	1.8
Anterior trunk	2	1		1			2	6	12	7.1
Back	27	5	4		2	3	2	17	60	35.5
Arms	20	5		5	3	2		2	37	21.9
Legs	6	8		2	1	4		17	38	22.4
Groin				4		4		6	14	8.3
Palms and soles								0	0	0
Traumatized areas					24				24	
Total lesions									193	
Total lesions in absence of trauma									169	
Average number of lesions per animal									21	

[a] Monkey No. 63 sustained a fractured tibia.

after the onset of illness. Crusts from lesions were collected for isolation of virus. Rectal temperatures were recorded daily with a thermistor tele-thermometer. Animals were not sedated during this procedure because it was noted that the sedative intended for use, phencyclidine hydrochloride,[1] depressed the body temperature.

Virus assay

Crust specimens were ground in McIlvain's buffer, treated with penicillin (200 IU per ml) and streptomycin (0.1 mg per ml), and cultured on the chorioallantonic membrane of 10-day-old embryonated chicken eggs.

Serological tests

Haemagglutination-inhibition (HI) tests were conducted using a microadaptation of the method described by Kempe (1964). A partially purified vaccinia haemagglutinin was used.

RESULTS

The variola major virus produced a mild exanthematous illness in the monkeys. In the last 24–48 hours of the incubation period, and when febrile, the animals were less aggressive, had decreased appetites and developed ecchymoses at sites of skin trauma. Occasional animals developed gingivorrhagia and blood-streaking in the faeces. Animals were febrile for 24–48 hours and at this time often appeared flushed, with red faces, had shaking chills, coughs and hoarseness. No facial or periorbital oedema was noted.

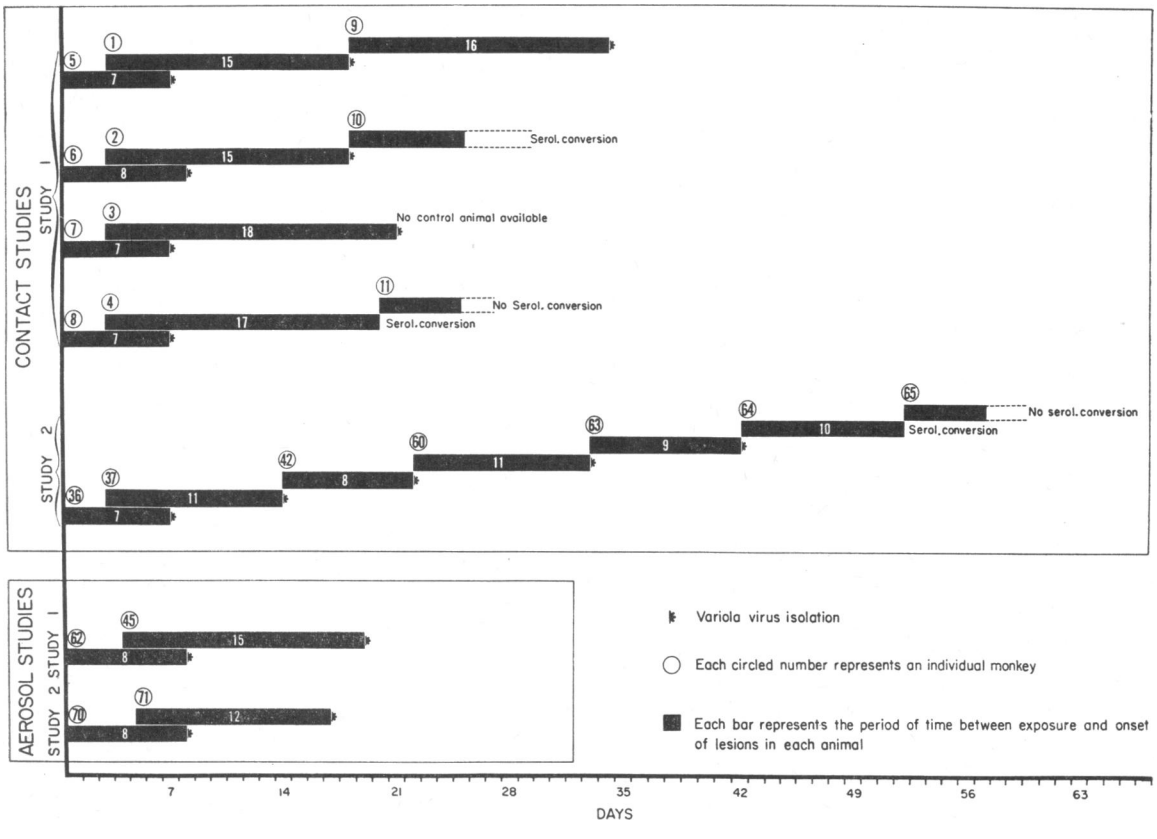
Rash appeared with onset of fever or within the following 24 hours; its onset was usually sudden. Animals No. 42 and No. 63 developed extensive papular exanthems in 1 and 5 hours, respectively, during the first day of fever. Lesions continued to appear over a 48-hour period. The papular lesions evolved through vesicular and pustular stages in 3–5 days and were more superficial than similar lesions in human cases of smallpox. When healed, they left shallow depigmented scars. The lesions in 2 animals were atypical, presenting as superficial ulcers with a thin exudate and no vesicle dome; however, variola virus was cultured in high titre (10^7 pock-forming units per ml).

The distribution of lesions was centrifugal with the greatest number and concentration of lesions occurring on the back, arms and legs; none were noted on the palms and soles of hands and feet (Table 1). Traumatized extremities, areas where restraints were applied, and areas of friction on animals maintained in restraining chairs showed increased numbers of lesions. The relative sparing of the face, hands and

[1] Sernylan; Parke, Davis and Co., Detroit, Mich., USA.

FIG. 2

GENERATIONS OF SMALLPOX TRANSMISSION IN *MACACA IRUS PHILIPPINENSIS*



feet differentiated the distribution of lesions in *Macaca irus* from the classical distribution of smallpox lesions in man.

Contact transmission of disease

In the first contact study, each of 4 inoculated animals (numbers 5, 6, 7, 8) transmitted infection to their cage-mates (numbers 1, 2, 3, 4, respectively). The inoculated monkeys developed pyrexia and an exanthematous eruption on the seventh or eighth day; lesions appeared on the cage-mate-control monkeys 15–18 days after exposure to the inoculated monkeys. Three of these animals (numbers 1, 2, 4) were then placed with new healthy cage-mates (numbers 9, 10, 11). Monkey No. 9 subsequently developed an exanthem, No. 10 converted serologically but did not develop lesions, and No. 11 did not develop lesions or convert serologically (Fig. 2). The diagnosis was established by isolating the virus in embryonated eggs and by serological conversion (Table 2).

The second contact study demonstrated serial transmission of smallpox through 6 passages (Fig. 2). Monkey No. 36, inoculated intranasally, transmitted the disease to healthy cage-mate No. 37. When lesions appeared on No. 37, this monkey was moved to a separate cage to expose, in turn, healthy animal No. 42. After developing lesions, No. 42 was placed with No. 60; then No. 60 with No. 63; and subsequently, No. 63 with No. 64. Variola was isolated from cutaneous lesions of all animals up to No. 64. No. 64 developed an exanthem like that seen in the other monkeys and converted serologically. However, variola virus was not isolated from it, and transmission of infection to monkey No. 65 was not successful.

TABLE 2
LABORATORY RESULTS DOCUMENTING SMALLPOX INFECTION IN THE MONKEYS USED IN TRANSMISSION STUDIES

Study No.	Animal No.	No. of lesions	Virus isolation [a]	HI antibody titre		
				S_1 [b]	S_2 [c]	Date S_2 [d]
1	5	Many	+	<5	320	20
	6	Many	+	<5	160	20
	7	Many	+	5	80	20
	8	Many	+	<5	160	20
2	1	Many	+	<5	160	11
	2	10–12	+	<5	640	11
	3	3	+	<5	160	8
	4	2	ND	<5	80	9
	9	4	+	<5	160	6
	10	0	ND	<5	10	21 days after exposure
	11	0	ND	<5	<5	21 days after exposure
2	36	Many	+	<5	320	10
	37	54	+	<5	320	9
	42	20	+	<5	160	20
	60	4	+	<5	80	15
	63	31	+	<5	320	11
	64	13	−	<5	320	13
	65	0	ND	<5	<5	21 days after exposure
3	62	13	+	<5	320	12
	45	Many	+	<5	128	14
4	70	6	+	<5	80	5
	71	51	+	<5	320	5

[a] ND = not done.
[b] Pre-inoculation serum.
[c] Serum obtained at end of study.
[d] The number of days between the appearance of a rash and the S_2 bleeding.

Aerosol transmission of disease

In the first aerosol transmission study, monkey No. 62 was inoculated intranasally and placed in the animal-holding chamber in the intake (upwind) chair. After a period of 72 hours, a control monkey, No. 45, was placed in the exposure (downwind) chair and remained in the chamber for the next 15 days. Eight days after inoculation, No. 62 developed fever and 13 lesions. Fifteen days after exposure to No. 62, monkey No. 45 developed fever and an extensive rash. Variola virus was isolated from lesions of both animals.

The second aerosol transmission study followed the same protocol as the first except that the control animal (No. 71) was exposed to the inoculated animal (No. 70) 96 hours after inoculation instead of after 72 hours. Monkey No. 70 developed fever and lesions 8 days after inoculation. Twelve days after exposure to No. 70, fever and extensive lesions were

noted on No. 71. Variola virus was isolated from lesions on both animals.

DISCUSSION

The clinical syndrome of smallpox in monkeys described in these studies corresponds to that reported in the literature (Hahon, 1961). The distribution of the rash with relative sparing of the head and face, the short incubation period, the mildness of the infection and rapid evolution of lesions, all differentiate simian smallpox from human smallpox. The successful transmission of smallpox in *Macaca irus* and maintenance of infection for 6 serial passages demonstrate that this species is capable of harbouring human strains of variola under experimental conditions. The species can, therefore, be considered a potential reservoir for smallpox. The transmission of infection both by contact and by aerosol exposure indicates that disease could be spread among wild monkeys.

At present there is no definite evidence that smallpox occurs in wild monkey populations. From reported outbreaks and epidemiological surveillance for poxvirus infection in captured monkeys, Arita & Henderson (1968) conclude that this phenomenon is rare, if it occurs at all. The epizootics reported by Anderson (1861) and Bleyer (1922) were not confirmed by laboratory testing; and Anderson (1861) described high mortality, extensive facial lesions and periorbital oedema in the afflicted monkeys. Smallpox causes only a very low mortality rate in monkeys, and facial oedema has not been noted in our studies, nor is it reported by Hahon (1961) in his review of simian smallpox. The description of monkeypox infection in captive monkeys presented by Von Magnus et al. (1959), Sauer et al. (1960), Peters (1966) and McConnell et al. (1964) may provide an alternate diagnosis for these epizootics of smallpox-like illness in monkeys. Monkeypox infection often produces extensive facial lesions with periorbital oedema, and fatal cases have been reported in some primate species (Peters, 1966).

Smallpox transmission has been studied for many years. Epidemiological investigations have implicated fomite and aerosol routes (WHO Expert Committee on Smallpox, 1964). Zuelzer (1874) observed that smallpox-infected materials contained in a wire basket and placed with healthy monkeys transmitted infection. Downie et al. (1965), using an impinger, isolated variola virus from expired air of only 5 out of 42 smallpox patients and concluded from these results that transmission by fomites may be more important than transmission by droplet nuclei. Variola has been isolated from the respiratory tracts of a human smallpox case (MacCallum et al., 1950) and experimentally infected monkeys (Hahon, 1961; Westwood et al., 1966). Our studies with aerosols ruled out transmission by ingestion of contaminated excreta or crusts from infected animals and demonstrated that intimate physical contact is not required for the transmission of disease. They show that variola can spread by the aerosol route.

Animals inoculated intranasally had an incubation period of 7–8 days which is similar to the incubation period described by Hahon (1961) for *Macaca irus* exposed to a variola aerosol. The interval between exposure and the appearance of lesions in animals exposed to infected cage-mates in the 4 studies averaged 10.8 days, with a range of 8–16 days. There was no correlation between the number of cutaneous lesions and the ability of an animal to transmit infection.

During our studies no increased virulence of the variola virus was observed after serial passage in *Macaca irus*. The smallpox infections appeared to die out in both contact transmission studies and the number of lesions on each succeeding monkey declined in the first contact study (Table 2). This was also noted in the second study until monkey No. 63 developed 31 lesions. This animal sustained a fractured tibia on the day it was placed with monkey No. 60 and 24 lesions appeared under a leg-splint. Monkey No. 63 subsequently transmitted smallpox to No. 64. Ricketts (1908) described increased density of smallpox lesions at sites of skin trauma. Whether a fractured long bone increases the susceptibility of a monkey to smallpox is not known. It is possible that the infection would have died out after monkey No. 60 if No. 63 had been a completely healthy animal.

Whenever primates are exposed to human epidemics of viral etiology, there may be transmission of infection. This has been suggested for smallpox (Bras, 1962), varicella (Heuschele, 1960) and measles (Meyer et al., 1962). Our studies have demonstrated that smallpox infection can be transmitted, and maintained, by susceptible monkeys and that it is theoretically possible for them to serve as a reservoir for variola virus. *Macaca irus* is found throughout Indonesia and the Philippines and inhabits many areas where smallpox is, or has been, endemic. In spite of its susceptibility to variola and its ability to transmit infection, no naturally occurring cases of smallpox have ever been reported in this species, nor

have cases of smallpox been reported in *Macaca mulatta*, a species that occurs throughout Asia, which is less susceptible than *Macaca irus* to experimental variola infection. Thus, at the present time there is no evidence that variola extends beyond human populations to these simian species.

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RÉSUMÉ

TRANSMISSION DE LA VARIOLE PAR CONTACT ET PAR L'INTERMÉDIAIRE D'AÉROSOLS CHEZ LE SINGE *MACACA IRUS*

On sait que le virus variolique est pathogène pour certaines espèces simiennes. Néanmoins, on ne dispose jusqu'à présent d'aucune donnée concernant la transmission naturelle de l'infection parmi les singes. Une série d'expériences a été effectuée sur des singes *Macaca irus* afin d'étudier la possibilité de la transmission de la variole majeure par contact et par l'intermédiaire d'aérosols et de vérifier si l'infection peut être maintenue par passages répétés chez cette espèce. Les critères de transmission de la maladie ont consisté en l'isolement du virus et en l'apparition d'anticorps spécifiques chez les animaux exposés.

Au cours de la première expérience, on a infecté quatre singes par inoculation intranasale de 2 ml de suspension virale ($2{,}5 \times 10^6$ unités formatrices de pustules par millilitre). Chaque animal a été ensuite placé dans une cage distincte avec un compagnon sain. Dans tous les cas, ce dernier a contracté l'infection. Trois des singes ainsi infectés par contact ont été encagés chacun avec un congénère sain. L'un de ceux-ci a présenté une éruption; chez le deuxième, on a décelé une conversion sérologique, sans lésions cutanées; le troisième n'a présenté ni lésions ni conversion sérologique. Au cours de la seconde expérience de transmission par contact, on a réussi à propager l'infection variolique par passages en série chez six singes.

Les expériences au moyen d'aérosols ont été réalisées sur deux singes placés à environ 1 mètre de distance dans un local isolé où circulait un courant d'air unidirectionnel. Lorsque le premier animal, installé en amont, a été infecté par inoculation intranasale, l'infection variolique s'est transmise à son compagnon après un délai de 12-15 jours.

Bien qu'aucune donnée épidémiologique ne permette d'affirmer l'existence de réservoirs simiens de la variole, il apparaît à la lumière de ces expériences que l'infection peut se transmettre au sein de certaines espèces. L'importance de ce problème en ce qui regarde l'éradication de la variole justifie de nouvelles recherches.

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